dispersed in the Waring Blendor using a fresh portion of cold 0.1 M Na_2HPO_4 and extracted an additional one hour. The dispersed slices are extracted with fresh cold 10% NaCl once daily for the three days. Finally, the dispersed slices are washed with water and swelled in 1 liter of 0.3% by volume acetic acid in the cold overnight. The swollen slices are dispersed in a Waring Blendor and the swollen, dispersed fibrils are filtered through a single layer of cheesecloth and then a double layer of cheesecloth to remove unswollen material.

The swollen fibrils are precipitated from the acid dispersion by the addition of 5% by weight sodium chloride. The fibrils are collected by centrifugation; neutralized with ammonia and washed free of chloride with water. The purified fibrils are again dispersed in 0.3% acetic acid, the concentration of collagen solids being 0.2%, and 0.0001% by weight of aureomycin is added.

Twenty-five milligrams of crystalline pepsin is added to 248 g. of the above dispersion and the mixture is incubated at 25° C. for 12 days. At the end of this time the fibrils disappear (phase contrast optics) leaving a solution of soluble collagen.

The soluble collagen was dialyzed against cold 1% NaCl for several days and then slowly warmed to room temperature. On warming fibrils precipitated out. The fibrils when examined in the electron microscope showed the characteristic 640 A. spacing of collagen. The yield of the fibrils based on hydroxyproline recovery was 74.7%.

Example II

A portion of split steer hide is frozen and sliced in the meat slicer. The slices are treated as in Example I and the purified fibrils are dispersed at a concentration of 0.2% in 0.3% acetic acid containing 0.0001% aureomycin. Fifty-seven mg. of crystalline pepsin is added to 566.5 g. of this dispersion. After six days at 25° C. fibrils can no longer be seen in the microscope (phase contrast). After dialysis against cold 1% NaCl and warming to room temperature collagen fibrils precipitate which showed the typical 640 A. spacing in the electron microscope. The yield of fibrils based on hydroxyproline recovery is 76.5%.

Example III

A portion of a split steer hide is frozen and sliced on the meat slicer. The slices are treated as in Example I and the purified fibrils are dispersed at a concentration of 0.3% in 0.3% acetic acid containing 0.0001% aureomycin and 0.01 M cysteine. To 100 mg. of this dispersion is added 10 mg. of a partially purified papain preparation containing 945 azocoll units per mg. After standing at 25° C. for 10 days, no fibrils can be seen in the microscope (phase contrast). Fibrils of soluble collagen are reconstituted by the addition of 5% by weight solid sodium chloride and neutralizing the acid with ammonia. Fibrils are collected by centrifugation, dialyzed against distilled water and freeze-dried. The yield of soluble collagen based on hydroxyproline recovery was 57.2%.

Example IV

To 100 mg. of the dispersion of collagen fibrils described in Example III is added 10 mg. of a partially purified preparation of bromelain with an activity of 182 azocoll units per mg. After standing at 25° C. for ten days, fibrils can no longer be seen in the microscope (phase contrast). Fibrils of soluble collagen are reconstituted by the addition of 5% by weight solid sodium chloride and neutralizing the solution with ammonia. Fibrils are collected by centrifugation, dialyzed against distilled water and freeze-dried. The yield of soluble collagen based on hydroxyproline recovery is 56.5%.

Example V

To 500 grams of the dispersion described in Example III is added 10 mg. of a partially purified preparation of

pinguinain with a density of 2367 azocoll units per mg. After standing at 25° C. for 4 days, no fibrils can be seen in the microscope (phase contrast). To this solution is added 1% by weight solid NaCl and the solution is neutralized with ammonia. A precipitate of collagen fibrils forms upon standing. The fibrils are collected by centrifugation, dialyzed against distilled water and freeze-dried. The yield of soluble collagen based on hydroxyproline recovery is 59.6%.

Example VI

A dispersion of collagen fibrils is prepared from the flexor tendons of the legs of steers as described in Example I. In its final form the dispersion in 0.3% acetic acid contains 0.2% collagen fibrills, 0.0001% aureomycin and 0.01 M cysteine. To 30 g. of this dispersion at 25° C. is added 0.30 ml. of an aqueous extract of crude ficin. The ficin extract is prepared by suspending 50 mg. of crude ficin in 5 ml. of water, stirring for ten minutes and then centrifuging. The supernatant liquid is filtered and the clear ficin extract used as described above.

After 10 days, no fibrils can be seen in the microscope (phase contrast). One percent by weight solid NaCl is added. After neutralization and standing at 25° C. overnight, the soluble collagen precipitates in the form of fibrils that are collected by centrifugation, dialyzed against distilled water and freeze-dried. The yield of soluble collagen based on hydroxy-proline recovery was 66.7%.

The reconstituted collagen fibrils obtained from the soluble depolymerized collagen may be dispersed in acid solution and extruded to form collagen strands by the procedure described in U.S. Patent No. 2,919,999. Solutions of depolymerized collagen may also be used as a binding substance in the manufacture of shaped collagen fiber masses as described in the Highberger Patents No. 2,934,446 and No. 2,934,447.

While the invention has been described in detail according to the preferred manner of carrying out the invention, it will be obvious to those skilled in the art, after understanding the invention, that changes and modifications may be made without departing from the spirit or scope of the invention, and it is intended in the appended claims to cover such changes and modifications.

I claim:

1. The method of producing soluble collagen monomers which comprises the steps of:

Treating finely divided native collagen with a dilute acid solution to swell the collagen fibrils;

Treating the acid swollen collagen fibrils with an elastase while maintaining the temperature between 0° C. and 30° C. until all of the swollen collagen fibrils dissolve; and,

Stopping the reaction at that point by removing the acid from solution.

2. The method of claim 1 wherein the acid is removed from solution by dialysis.

3. The method of claim 1 wherein the acid is removed from solution by neutralization.

4. The method of claim 1 wherein the elastase is pepsin.

5. The method of claim 1 wherein the elastase is papain.

6. The method of claim 1 wherein the elastase is bromelain.

7. The method of claim 1 wherein the elastase is pinguinain.

8. The method of claim 1 wherein the elastase is ficin.

References Cited in the file of this patent

UNITED STATES PATENTS

2,973,302 Bloch et al. ---------- Feb. 28, 1961

OTHER REFERENCES

Gustavson: "The Chemistry and Reactivity of Collagen," Academic Press Inc., New York, 1956, pages 186, 187 and 260 and 271. (Copy in Div. 63.)